

Heart failure subtypes and thromboembolic risk in patients with atrial fibrillation:

Siller-Matula, Jolanta M.; Pecun, Ladislav; Patti, Giuseppe; Lucerna, Markus; Kirchhof, Paulus; Lesiak, Maciej; Huber, Kurt; Verheugt, Freek W. A.; Lang, Irene M.; Renda, Giulia; Schnabel, Renate B.; Wachter, Rolf; Kotecha, Dipak; Sellal, Jean-Marc; Rohla, Miklos; Ricci, Fabrizio; De Caterina, Raffaele; TEAM in AF group

DOI:

[10.1016/j.ijcard.2018.04.093](https://doi.org/10.1016/j.ijcard.2018.04.093)

License:

Creative Commons: Attribution-NonCommercial-NoDerivs (CC BY-NC-ND)

Document Version

Peer reviewed version

Citation for published version (Harvard):

Siller-Matula, JM, Pecun, L, Patti, G, Lucerna, M, Kirchhof, P, Lesiak, M, Huber, K, Verheugt, FWA, Lang, IM, Renda, G, Schnabel, RB, Wachter, R, Kotecha, D, Sellal, J-M, Rohla, M, Ricci, F, De Caterina, R & TEAM in AF group 2018, 'Heart failure subtypes and thromboembolic risk in patients with atrial fibrillation: The PREFER in AF - HF substudy', *International Journal of Cardiology*. <https://doi.org/10.1016/j.ijcard.2018.04.093>

[Link to publication on Research at Birmingham portal](#)

General rights

Unless a licence is specified above, all rights (including copyright and moral rights) in this document are retained by the authors and/or the copyright holders. The express permission of the copyright holder must be obtained for any use of this material other than for purposes permitted by law.

- Users may freely distribute the URL that is used to identify this publication.
- Users may download and/or print one copy of the publication from the University of Birmingham research portal for the purpose of private study or non-commercial research.
- User may use extracts from the document in line with the concept of 'fair dealing' under the Copyright, Designs and Patents Act 1988 (?)
- Users may not further distribute the material nor use it for the purposes of commercial gain.

Where a licence is displayed above, please note the terms and conditions of the licence govern your use of this document.

When citing, please reference the published version.

Take down policy

While the University of Birmingham exercises care and attention in making items available there are rare occasions when an item has been uploaded in error or has been deemed to be commercially or otherwise sensitive.

If you believe that this is the case for this document, please contact UBIRA@lists.bham.ac.uk providing details and we will remove access to the work immediately and investigate.

Heart failure subtypes and thromboembolic risk in patients with atrial fibrillation: the PREFER in AF - HF substudy

Jolanta M. Siller-Matula^{1,2} MD PhD, Ladislav Pecen³ PhD, Giuseppe Patti⁴ MD, Markus Lucerna⁵ PhD, Paulus Kirchhof^{6,7} MD, Maciej Lesiak² MD, Kurt Huber⁸ MD, Freek W.A. Verheugt⁹ MD, Irene M. Lang¹ MD, Giulia Renda¹⁰ MD PhD, Renate B. Schnabel^{11,12} MD, Rolf Wachter^{13, 14} MD, Dipak Kotecha⁶ MD PhD, Jean-Marc Sella¹⁵ MD, Miklos Rohla¹⁶ MD, Fabrizio Ricci¹⁰, MD, , and Raffaele De Caterina¹⁰ MD PhD;
TEAM in AF group

¹ Department of Cardiology, Medical University of Vienna, Vienna, Austria;

² ^{1st} Department of Cardiology, Poznan University of Medical Sciences, Poland;

³ Institute of Informatics, Academy of Sciences of Czech Republic, Prague, Czech Republic;

⁴ Department of Cardiovascular Sciences, Campus Bio-Medico University of Rome, Italy;

⁵ Daiichi Sankyo Europe, Munich, Germany;

⁶ Institute of Cardiovascular Sciences, University of Birmingham and SWBH and UHB NHS Trusts, Birmingham, UK;

⁷ AFNET, Münster, Germany;

⁸ 3rd Medical Department, Cardiology and Intensive Care Medicine, Wilhelminen Hospital, and Sigmund Freud University, Medical School, Vienna, Austria;

⁹ Emeritus Professor of Cardiology, Amsterdam, The Netherlands;

¹⁰ G. d'Annunzio University of Chieti and Center of Excellence on Aging, CeSI-Met, Italy;

¹¹ Department for General and Interventional Cardiology, University Heart Center Hamburg, Hamburg, Germany;

¹² German Center for Cardiovascular Research (DZHK) partner site Hamburg/Kiel/Lübeck, Hamburg, Germany;

¹³ Clinic for Cardiology and Pneumology, University Medical Center Göttingen, Göttingen, Germany;

¹⁴ German Center for Cardiovascular Research, partner site, Göttingen, Germany.

¹⁵ Department of Cardiology, University Hospital Nancy, France;

¹⁶ 3rd Medical Department, Cardiology and Intensive Care Medicine, Wilhelminen Hospital, Vienna, Austria.

Word count: 3500

SHORT TITLE: *HF type in AF predicts thromboembolic risk*

CORRESPONDENCE:

Jolanta Siller-Matula, Medical University of Vienna, Vienna, Austria; Währinger Gürtel 18-20, 1090 Vienna, Austria, Tel: 0043 1 40400 46140, Fax: 0043 1 40400 42160;

Email: jolanta.siller-matula@meduniwien.ac.at

Or

Raffaele De Caterina, MD, PhD; University Cardiology Division, "G. d'Annunzio" University – Chieti; Email: rdecafer@unich.it

ABSTRACT

BACKGROUND and OBJECTIVES: To assess thromboembolic and bleeding risks in patients with heart failure (HF) and atrial fibrillation (AF) according to HF type.

METHODS: We analyzed 6,170 AF patients from the Prevention of thromboembolic events - European Registry in Atrial Fibrillation (PREFER in AF), and categorized patients into: HF with reduced left-ventricular ejection fraction (HFrEF; LVEF<40%); mid-range EF (HFmrEF; LVEF: 40-49%); lower preserved EF (HFpEF; LVEF: 50-60%), higher preserved EF (HFHpEF; LVEF>60%), and no HF. Outcomes were ischemic stroke, major adverse cardiovascular and cerebral events (MACCE) and major bleeding occurring within 1-year.

RESULTS: The annual incidence of stroke was linearly and inversely related to LVEF, increasing by 0.054% per each 1% of LVEF decrease (95% CI: 0.013%-0.096%; p=0.031). Patients with HFHpEF had the highest CHA₂DS₂-VASc score, but significantly lower stroke incidence than other HF groups (0.65%, compared to HFpEF 1.30%; HFmrEF 1.71%; HFrEF 1.75%; trend p=0.014). The incidence of MACCE was also lower in HFHpEF (2.0%) compared to other HF groups (range: 3.8-4.4%; p=0.001). Age, HF type, and NYHA class were independent predictors of thromboembolic events. Conversely, major bleeding did not significantly differ between groups (p=0.168).

CONCLUSION: Our study in predominantly anticoagulated patients with AF shows that, reduction in LVEF is associated with higher thromboembolic, but not higher bleeding risk. HFHpEF is a distinct and puzzling group, featuring the highest CHA₂DS₂-VASc score but the lowest residual risk of thromboembolic events, which warrants further investigation.

Key words: atrial fibrillation; heart failure; stroke; ejection fraction; bleeding.

Acknowledgement: This analysis of the PREFER in AF registry was initiated by the Thrombosis Exchange Meeting in AF, TEAM in AF, funded and sponsored by Daiichi-Sankyo Europe.

Abstract count: 234

Main text count: 3987

INTRODUCTION

Heart failure (HF) is a clinical syndrome caused by structural and/or functional cardiac abnormalities, which results in reduced cardiac output and/or elevated intracardiac pressures [1]. In recent times, HF has been classified broadly into two groups, mainly based on the measurement of left ventricular ejection fraction (LVEF): HF with reduced EF (HFrEF, EF<50) and HF with preserved EF (HFpEF, EF \geq 50) [1, 2]. It has been estimated that approximately half of the patients with HF have HFpEF [2-6]. The European Society of Cardiology (ESC) has recently introduced a new subgroup of HF, defined as HF with mid-range EF (HFmrEF, EF 40-49%). A main shortcoming of the recent HF classification is that current knowledge about HFpEF and HFmrEF is limited, and is based on evidence mostly derived from retrospective observational cohort studies or post-hoc analyses of randomized trials [1, 2].

Atrial fibrillation (AF) and HF are tightly inter-connected entities [7-10]. Regardless of which condition arises first, the coexistence of these diagnoses confers substantially increased cardiovascular morbidity and mortality [11, 12]. HF and AF, jointly or in isolation, are likely to dominate the next era in cardiovascular disease epidemiology, in terms of prevalence, incidence, morbidity, mortality and healthcare expenditure [13-16]. Therefore, understanding predictors of outcome in AF patients according to different HF subtypes is of major clinical importance. Furthermore, the new reclassification of HF types introduced in the 2016 ESC guidelines [1] calls for a reappraisal of the thromboembolic and hemorrhagic risk stratification across different HF subtypes. To address these issues, we report on the HF sub-study of the Prevention of Thromboembolic Events European Registry in Atrial Fibrillation (PREFER in AF).

METHODS

PREFER in AF was a prospective, real-world registry on 7,228 AF patients from 461 hospitals and 7 European countries (Austria, France, Germany, Italy, Spain, Switzerland and the United Kingdom). Inclusion criteria were: age ≥ 18 years; at least one episode of AF in the previous one year, as demonstrated by an electrocardiogram or by an implanted pacemaker/defibrillator; and signed informed consent to be part of the study, mostly conducted in cardiology centers [17]. The first patient was included in January 2012, with the last follow-up visit being performed in January 2014. There were no explicit exclusion criteria. The study design included a baseline visit at the time of patient recruitment, and a clinical follow-up evaluation at 1 year. In this investigation we only included patients with data available from both the baseline and the 1-year follow-up visits. Only documented events were considered as relevant outcome measures, with any event occurring after the baseline assessment. The study design has been published [17, 18] and the protocol was approved by each local-site Ethics Committee. The registry was sponsored by Daiichi Sankyo Europe GmbH (Munich, Germany) via a contract research organization (SSS International Clinical Research GmbH – Munich, Germany) coordinating various local national contract research organizations.

Definitions and endpoints

The primary efficacy endpoint of this analysis was ischemic stroke. Secondary endpoints were i) the composite of major adverse cardiovascular and cerebral events (MACCE: stroke, systemic embolism, myocardial infarction and acute coronary syndrome), ii) the composite of thromboembolic events (stroke/transient ischemic attack (TIA)/arterial embolism (AE)), iii) death and iv) major bleeding occurring within 1 year of follow-up.

Stroke was defined as the abrupt onset of a focal neurologic deficit, generally distributed in the territory of a single brain artery (including the retinal artery), and not attributable to an identifiable non-vascular cause (i.e., brain tumor or trauma). The deficit had to be either characterized by symptoms lasting >24 hours or causing death within 24 hours of symptom onset. The stroke definition used in the ENGAGE-AF TIMI 48 study and in our study reflects the *Statement for Healthcare Professionals From the American Heart Association/American Stroke Association* that incorporates the *World Health Organization (WHO)* definition of stroke [19]. TIA was defined as a focal neurologic deficit associated with symptoms lasting <24 hours.

Systemic embolic event (SEE) was defined as an abrupt episode of arterial insufficiency with clinical or radiologic documentation of arterial occlusion in the absence of other likely mechanisms (e.g., atherosclerosis, instrumentation); venous thromboembolism and pulmonary embolism were also included in this outcome measure.

Acute coronary syndrome was defined as a myocardial infarction or unstable angina. Myocardial infarction (MI) was defined according to the latest version of the Universal Definition [20]. Unstable angina was defined by specific clinical findings of prolonged (>20 minutes) angina at rest; new onset of severe angina; angina that is increasing in frequency, longer in duration, or lower in threshold; or angina that occurs after a recent episode of MI, always in the absence of biochemical evidence of myocardial damage according to locally used troponin T or I tests [21].

Major bleeding was defined as fatal bleeding and/or bleeding into a critical organ (intracranial, intraspinal, intraocular, retroperitoneal, intraarticular or pericardial, or intramuscular with compartment syndrome) and/or clinically relevant bleeding with a hemoglobin drop ≥ 2 g/dL; this is consistent with the definition of major bleeding from the *International Society on Thrombosis and Haemostasis* [4].

HF definition and classification

Treating physicians at the enrolling sites made a clinical diagnosis of HF (HF with reduced or preserved LVEF as per HF guidelines available at the time of inclusion), without any centralized adjudication of the diagnosis. Likewise, treating physicians included data on the EF, derived from echocardiography based on the Simpson's method, without a centralized adjudication and verification. As a second step, we grouped patients with HF into HF with reduced EF (HFrEF; EF<40%); HF with mid-range EF (HFmrEF; EF: 40-49%); and HF with preserved ejection fraction (HFpEF; EF>50%), based on the most recent ESC guidelines [1]. Thirdly, as an exploratory analysis, we further subdivided the HFpEF cohort into HF with lower preserved ejection fraction (HFLpEF ; EF: 50-60%) and HF with higher preserved ejection fraction (HFHpEF; EF>60%).

Statistics

We here report categorical variables as absolute and percent frequencies (n, %). For each continuous variables, we report the mean, median, standard deviation or 95% confidence intervals (CI), as appropriate.

We performed a complete case analysis and assumed that missing data were missing at random. We performed statistical comparisons with the t test, the Mann Whitney U test or the Chi²-test, as appropriate. We then calculated odds ratios (OR) for independent predictors of thromboembolic events in HF patients by multivariable logistic regression, where predictors and adjusting factors were included in the model. The composite of thromboembolic events (yes/no) was the dependent variable, whereas the following factors were included into the model as independent variables: EF, HF subtype (HFrEF/HFmrEF/HFLpEF/HFHpEF), LVEF per 10% decrease, New York Heart Association

(NYHA) class, anticoagulation treatment, CHA₂D₂VASc score (applied as indicated in the AF guidelines [22], where 1 point for congestive HF was given in patients with LV dysfunction and / or congestion at the time point of inclusion), body mass index (BMI), smoking. We report ORs, 95% confidence intervals (CIs) and the corresponding p value for such analyses.

A post hoc power calculation has revealed a power of 84% for the comparison of the composite of MACCE event rates between the groups with a two-sided p value <0.05.

All analyses are to be intended as descriptive/exploratory, and therefore no adjustment for multiple testing was done. All statistical analyses were performed using SAS, version 9.4 (Cary, North Carolina, USA), with a two-tailed significance value of 0.05.

RESULTS

The flow of patients through the PREFER in AF-HF substudy is shown in **Figure 1**. Out of 7,228 patients enrolled in the PREFER in AF Registry, 6,170 had baseline and 1-year follow-up visits, complete data on the incidence of thromboembolic events, and information on the HF diagnosis. Of these, 4,571 had no HF and 1,599 had a HF diagnosis. Of these latter, 458 had HFrEF, 525 had HFmrEF and 616 had HFpEF. Among patients with HFpEF, 308 were classified with HFLpEF, and 308 with HFHpEF.

The distributions of demographic and clinical features according to HF type are indicated in **Tables 1 and 2**. Among patients with HF, patients with HFrEF were more often male, smokers and with a younger age, more often with a history of vascular disease and of chronic kidney disease. In contrast, patients with HFHpEF were more often female, with a higher age and a higher mean systolic blood pressure as compared with other HF groups (**Table 1**).

We found the highest CHA₂DS₂-VASc score in patients with HFHpEF (mean 4.7) and the lowest in HFrEF (4.1) ($p<0.0001$; **Table 2**). Concordantly, 99% of HFHpEF patients had a clear indication for oral anticoagulation (OAC; CHA₂DS₂-VASc ≥ 2) compared with 95% in patients with HFrEF ($p<0.001$; **Supplement Figure 1S; Table 2**). The proportion of patients without OAC treatment despite indication (CHA₂DS₂-VASc ≥ 2) was lowest in the HFHpEF subgroup (6%) as compared to other HF subgroups (13% in each HFLpEF, HFmrEF and HFrEF, and 15% in no HF group; $p=0.0004$; **Supplement Figure 1S**). Of note, due to the time period in which PREFER in AF was performed, the penetration of NOACs was $<10\%$, and was highest in HFHpEF as compared to other groups (9.4% in HFHpEF; 5.5% in HFLpEF; 5.5% in HFmrEF; 4.6% in HFrEF; $p=0.026$; **Table 1**). The frequency of paroxysmal AF was in the same range in HF patients (18-21%) and was highest in no HF patients (31%; $p<0.001$ for trend).

Clinical outcomes

Patients with any diagnosis of HF had a higher incidence of stroke as compared to patients without HF (1.3% vs 0.6% year; respectively; $p=0.007$). Despite the highest CHA₂DS₂-VASc score, the yearly incidence of stroke was only 0.65% in HFHpEF, significantly lower than in other HF subgroups (1.30% in HFLpEF; 1.71% in HFmrEF; 1.75% in HFrEF; $p=0.014$; **Figure 2A**), and increased by 0.054% per 1% of EF decrease (95% CI: 0.013%-0.096%; $p=0.031$; **Figure 2B**). Also, in anticoagulated HF patients the incidence of ischemic stroke increased by 0.030% per each 1% of EF decrease (95% CI: 0.011%-0.048%; $p=0.003$). Stroke incidence was comparable between HFHpEF and no-HF groups (**Figure 2A**; $p=0.9746$).

Patients with any diagnosis of HF had a higher incidence of MACCE as compared to patients without HF (3.8% vs 2.1% per year; respectively; $p<0.001$). The yearly incidence of MACCE was lower in HFHpEF (2.0%) compared with other HF subgroups (4.2% in HFLpEF;

3.8% in HFmrEF; 4.4% in HFrEF; $p=0.001$; **Supplement Figure 2S**). MACCE incidence was comparable between HFHpEF and no-HF groups (**Supplement Figure 2S**; $p=0.9234$).

The incidence of major bleeding was higher in patients with HF as compared to patients without HF (3.6% vs 2.5% per year, respectively, $p=0.01$). There were no statistical differences in the incidences of the composite of major bleeding across the subgroups (trend $p=0.161$; **Supplement Figure 3S**). Of note, the incidence of major bleeding events according to bleeding type (intracranial bleeding (ICB), gastrointestinal (GI), other threatening major bleeding) was similar between HF groups, as well as in the entire study population ($p>0.05$; **Supplement Table 1S**).

Patients with any diagnosis of HF had a higher incidence of death as compared to patients without HF (6.0% vs 1.9% year; respectively; $p<0.001$). Death rate increased with decreasing EF, and was highest in the HFrEF subgroup (HFrEF: 7.2%, HFmrEF: 6.5%, HFpEF: 5.2%, HFHpEF: 4.2%, no HF: 1.9%; $p<0.001$). Death incidence was higher in patients with HFHpEF vs no-HF ($p=0.005$).

There were no differences in the outcomes in respect to AF type in the overall population as well as there was no interaction between AF type, different HF subtypes and clinical outcomes (paroxysmal vs non-paroxysmal; $p>0.05$ for all comparisons).

Predictors of thromboembolic risk in HF patients

The multivariable regression analysis identified 3 factors at baseline independently associated with thromboembolic events: HF subgroup ($p=0.007$), NYHA class ($p<0.001$) and age ($p=0.029$). Among different HF subgroups, the highest odds for the composite of thromboembolic events were in HFmrEF patients (OR: 3.10; 95% CI: 1.12-8.56). The following estimates were calculated for the NYHA class (OR per 1 increasing NYHA point:

2.92; 95% CI: 1.60-5.30) and age (OR per 1 year of age: 1.04; 95% CI: 1.00-1.08). While, as expected, the severity of HF symptoms (NYHA class III-IV) increased with decreasing EF (**Supplement Figure 4S.A**), NYHA classes III-IV (severe HF) were associated with a higher incidence of thromboembolic events as compared to NYHA classes I-II (mild to moderate HF), which was 12.3% and 12.5% in patients with HFmrEF and HFLpEF, respectively ($p < 0.05$ for between group comparison; **Supplement Figure 4S.B**).

Performance of the CHA2DS2-Vasc score in the prediction of stroke

In patients with no HF the CHADS-VASc score ≥ 2 performed well in terms of prediction of stroke (OR: 1.70, 95%CI: 1.34-2.16, $p < 0.0001$; AUC: 0.73, 95%CI: 0.62-0.83). In contrast, for HF patients its performance decreased with increasing HF severity. In the overall HF population the CHADS-VASc score ≥ 2 was not predictive of stroke (OR: 1.03, 95%CI: 0.79-1.33, $p = 0.85$; AUC: 0.50, 95%CI: 0.41-0.60). When each of the HF groups were analysed separately, the AUC for the CHADS-VASc score ≥ 2 decreased with the decreasing EF (HFHpEF AUC: 0.628, 95%CI: 0.03-1.00; HFLpEF AUC: 0.54, 95%CI: 0.36-0.72; HFmrEF AUC: 0.46, 95%CI: 0.33-0.58; HFrEF AUC: 0.44, 95%CI: 0.25-0.62) and was predictive for stroke in none of the HF subgroups in the logistic regression model.

Sensitivity analyses

When patients without OAC treatment (ranging from 6% to 19% depending on the group) were excluded from each of the analyses, the magnitude and the direction of the estimates remained unchanged (data not shown).

DISCUSSION

The central findings of the PREFER in AF – HF substudy investigating the association between HF type and thromboembolic events in this mainly anticoagulated cohort of AF patients in real-life clinical conditions under registry setting are as follows: i. the subtype of HF predicts the residual risk of thromboembolic events, with an inverse association between LVEF and hard thromboembolic endpoints, such as ischemic stroke and MACCE; ii. in HF patients, in addition to EF and age, the NYHA class is a strong and independent predictor of thromboembolic events; iii. HF patients with HFLpEF and HFHpEF represent quite distinct populations, which also differ in terms of thromboembolic risk; iv. mortality increases with decreasing EF, and was highest in the HFrEF subgroup.

The definition of HFpEF is difficult, which can be illustrated by the various classifications proposed by experts and by disparate inclusion criteria of clinical trials [2], which led to heterogeneity of HFpEF patients recruited into most studies and registries. Such difficulties in classification also affect our study. Even for the key diagnostic criterion, EF, different cut-offs have been used across trials and registries on HFpEF (ranging from $\geq 40\%$ to $\geq 55\%$), of which most were applied in a post-hoc manner [12]. The validity and reproducibility of estimating systolic and diastolic function in the context of AF has also been questioned [23]. Therefore, the available evidence addressing the issue of stroke risk among different HF subtypes, is based on studies using different inclusion and exclusion criteria, end point assessments, sample sizes, patient populations and characteristics. This is mirrored in the substantial statistical and clinical heterogeneity across studies [12]. Many such limitations also apply to our study, but still the present findings add important insights into the discussion on the risk of stroke in AF across HF subtypes.

Although the prognosis of all patients with HF is poor, HFpEF has been postulated to be more benign as compared to HFrEF [8]. Indeed, considering the risk of

thromboembolism in the presence of AF, HFrEF was associated with the highest incidence of adverse events such as ischemic stroke, MACCE and death in our analysis, which confirms the findings of other prospective studies, such as the Studies of Left Ventricular Dysfunction (SOLVD), the Survival and Ventricular Enlargement (SAVE), the Northern Manhattan Study (NOMASS), or the Apixaban for Reduction in Stroke and Other Thromboembolic Events in Atrial Fibrillation (ARISTOTLE) trials [24-27], showing that EF is an independent risk factor for stroke or systemic embolism [28]. In contrast, several other studies as the Loire Valley Atrial Fibrillation Project [29], the Atrial Fibrillation Clopidogrel Trial With Irbesartan for Prevention of Vascular Events (ACTIVE) trial in patients not randomized to oral anticoagulation [30], or a meta-analysis of cohort studies and post-hoc sub-group analyses of randomized trials in AF patients indicated no difference in the risk of stroke between HFrEF vs HFpEF groups [12]. A possible explanation for these differences is the circumstance that in some studies patients were not or rarely anticoagulated and in the meta-analysis 50% of patients were anticoagulated [12], as compared to 90% in our study. Therefore, our study focused on the residual risk of stroke in the majority of our patients, and such residual risk is known to be affected by the type of AF and the presence of HF [31]. A third possible explanation for the inconsistent results between our study and the previous meta-analysis is the fact that the definitions of HFpEF were very heterogenous and applied only post-hoc for the majority of included studies [2, 12]. A key step that allowed more insightful information on the risk of stroke in the HFpEF population in our study was to subdivide such patients into HFLpEF and HFHpEF.

In parallel with the risk of stroke, an association between HF subtypes and mortality has been previously described [12], indicating a 1.24-fold higher risk of death for HFrEF as compared with HFpEF. Thus, we confirm this finding in the PREFER in AF - HF substudy.

A true central finding of the present analysis is the uniqueness of mainly anticoagulated AF patients with HFHpEF, apparently representing a distinct patient

population. Such patients are characterized by the highest CHA₂DS₂-VASc score, but also, surprisingly, by the lowest incidence of thromboembolic events. This association was also confirmed after exclusion of patients without a proper anticoagulant treatment. Furthermore, it is crucial to note that splitting the HFpEF into two groups, those with HFLpEF and HFHpEF, revealed a statistically significant difference in the rate of thromboembolic events between such groups. Indeed, the incidence of ischemic stroke or MACCE was half in HFHpEF compared with HFLpEF. Therefore, distinguishing a group of HFHpEF in our study might underline the complex pathophysiology and heterogeneity of the HFpEF syndrome. In contrast, we have found no significant differences in the rates of thromboembolic events between HFrEF and HFmrEF.

Guidelines encourage the use of the CHA₂DS₂-VASc score only for the decision making on initiation of anticoagulant treatment in AF patients [22]. Patients with a CHA₂DS₂-VASc score of ≥ 2 , represent 85% of AF population being at moderate-to-high risk of thrombotic events, and who should benefit from anticoagulation. Importantly, the CHA₂DS₂-VASc score has never been established and is not recommended as a tool for predicting thromboembolism in patients already on anticoagulation, as it was the case in 90% of patient population included in this study. Interestingly, CHA₂DS₂-VASc score predicted stroke only in patients without HF in our study. In patients with HF irrespectively of the HF subtype, CHA₂DS₂-VASc score was not a predictor of residual risk of stroke. Moreover, the performance of CHA₂DS₂-VASc score to predict stroke in anticoagulated HF patients decreased with decreasing EF, which might confirm our results that EF is strongly associated with a residual risk of stroke in HF patients.

It has been reported that measures of cardiac performance, such as the LVEF, correlate poorly with HF symptoms (e.g., NYHA class) [32]. Accordingly, some patients with HFrEF may be asymptomatic, whereas some patients with HFpEF may have severe

dyspnea, as also demonstrated in our analysis. Our study, however, also showed that NYHA functional class was associated with thromboembolic events, independent of the HF type and other factors, increasing the odds for thromboembolism by 2.9 per 1 increasing NYHA point. Our observation that the clinical severity of HF in AF has independent and direct prognostic implications, confirms previous findings in the overall HF population [28]. Concordantly, the incidence of stroke was 3-fold higher in patients with more severe HF (median NYHA class 3.4) in the Prospective Randomized Milrinone Survival trial (PROMISE trial) as compared to patients with mild HF (median NYHA class 1.7) in the Studies of Left Ventricular Dysfunction (SOLVD trial) [33].

Importantly, our study design differs from a number of previously published studies on association between HF type and AF. Whereas in our analysis all patients had AF and 39% were also diagnosed with HF, other studies focused on patients who all had been diagnosed with HF, and some had also AF (prevalence ranging from 17% to 65%) [34-36]. Based on this assumption, we tested the incidence of thromboembolism in AF patients according to HF diagnosis and type. In contrast, other analyses focused on thromboembolic risk in HF patients according to rhythm disorder: AF vs no AF. Whereas results of both types of studies yield complementary information, such analyses are not interchangeable and a direct comparison of results is not feasible.

Study strengths and limitations

The strengths of our study include its focus on different types of HF, including the recent HF classification by the ESC, the large number (>6,000) of patients included, complete 1-year follow-up data on the incidence of thromboembolic events, and detailed sensitivity analyses with a focus on a new group of HFHpEF. Our study also adds data on the distribution of OAC use between the HF groups, predictors of thromboembolic events,

and the incidence of hard ischemic outcomes (such as ischemic stroke and MACCE) in each of the HF groups, as well as in relation to EF.

A major shortcoming of this study is the fact that the HF diagnosis (HFrEF or HFpEF, based on the available guidelines at the time of inclusion) was made by a treating physician at the study site and was not independently verified or adjudicated. **Moreover, diagnostic criteria for HF changed over time.** Due to the missing data on the levels of the brain natriuretic peptides (BNP), we could not verify the HF diagnosis. However, we performed a sensitivity analysis for a subgroup of patients with HFmrEF and HFpEF and available echocardiographic data recommended by the current guidelines for the HF diagnosis (left atrial enlargement, left ventricular hypertrophy), which confirmed the direction and the magnitude of the main study estimates. A second major shortcoming is here the use of echocardiographic assessment of the EF, known to be quite variable among operators and even within the single operator [37]. **Additionally, we are lacking information whether adjustment for heart rate at the time of echo was performed, which might also influence the assessment of EF.** EF derived by echocardiography with the modified Simpson formula was reported by the investigators and was not adjudicated. Much more accurate estimates of the EF are provided by magnetic resonance imaging [38], but such data were not available. The registry also did not provide data on parameters of left ventricular volumes or diastolic function, which have been shown to be potentially valuable in AF patients [23]. Moreover, imaging modalities were not adjudicated to ensure uniformity. Therefore, our study certainly lacks the standardization of HF diagnosis and of the EF measurements, and there is a risk of residual confounding. The registry also did not provide information on the underlying disease leading to HF. Mortality data were provided by the study centers following the final completion of the study, so that such data might be incomplete. Additional limitation is that the distinction between the two categories of HF with pEF (HFHpEF and HFLpEF) was a

part of post-hoc exploratory analyses. We fully acknowledge such limitations. This is however the first report in the literature describing a possible heterogeneity in the risk of stroke and bleeding within the broad category of patients with preserved EF, and calls for an independent validation of such data in independent cohorts.

CONCLUSION

In mainly anticoagulated patients with AF, the subtype of HF predicts the thromboembolic risk: patients with HFHpEF apparently represent a distinct patient population, with the highest CHA₂DS₂-VASc score but the lowest incidence of thromboembolic events. AF with HFrEF is associated with most severe adverse events, such as ischemic stroke and MACCE. HFHpEF with AF had a comparable thromboembolic risk to controls without HF but relatively high risk of bleeding. If independently confirmed, HFHpEF should be further inspected in future studies as possibly distinct from HFLpEF.

REFERENCES

- [1] Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC) Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016;37:2129-200.
- [2] Senni M, Paulus WJ, Gavazzi A, Fraser AG, Diez J, Solomon SD, et al. New strategies for heart failure with preserved ejection fraction: the importance of targeted therapies for heart failure phenotypes. *Eur Heart J*. 2014;35:2797-815.
- [3] Badheka AO, Rathod A, Kizilbash MA, Bhardwaj A, Ali O, Afonso L, et al. Comparison of mortality and morbidity in patients with atrial fibrillation and heart failure with preserved versus decreased left ventricular ejection fraction. *Am J Cardiol*. 2011;108:1283-8.
- [4] Schulman S, Kearon C. Definition of major bleeding in clinical investigations of antihemostatic medicinal products in non-surgical patients. *J Thromb Haemost*. 2005;3:692-4.
- [5] Kotecha D, Lam CS, Van Veldhuisen DJ, Van Gelder IC, Voors AA, Rienstra M. Heart Failure With Preserved Ejection Fraction and Atrial Fibrillation: Vicious Twins. *J Am Coll Cardiol*. 2016;68:2217-28.
- [6] Kitzman DW, Little WC, Brubaker PH, Anderson RT, Hundley WG, Marburger CT, et al. Pathophysiological characterization of isolated diastolic heart failure in comparison to systolic heart failure. *JAMA*. 2002;288:2144-50.
- [7] Anter E, Jessup M, Callans DJ. Atrial fibrillation and heart failure: treatment considerations for a dual epidemic. *Circulation*. 2009;119:2516-25.
- [8] Cuadrado-Godia E, Ois A, Roquer J. Heart failure in acute ischemic stroke. *Current cardiology reviews*. 2010;6:202-13.
- [9] Maisel WH, Stevenson LW. Atrial fibrillation in heart failure: epidemiology, pathophysiology, and rationale for therapy. *Am J Cardiol*. 2003;91:2D-8D.
- [10] Zakeri R, Chamberlain AM, Roger VL, Redfield MM. Temporal relationship and prognostic significance of atrial fibrillation in heart failure patients with preserved ejection fraction: a community-based study. *Circulation*. 2013;128:1085-93.
- [11] Mamas MA, Caldwell JC, Chacko S, Garratt CJ, Fath-Ordoubadi F, Neyses L. A meta-analysis of the prognostic significance of atrial fibrillation in chronic heart failure. *Eur J Heart Fail*. 2009;11:676-83.
- [12] Kotecha D, Chudasama R, Lane DA, Kirchhof P, Lip GY. Atrial fibrillation and heart failure due to reduced versus preserved ejection fraction: A systematic review and meta-analysis of death and adverse outcomes. *Int J Cardiol*. 2016;203:660-6.
- [13] Colilla S, Crow A, Petkun W, Singer DE, Simon T, Liu X. Estimates of current and future incidence and prevalence of atrial fibrillation in the U.S. adult population. *Am J Cardiol*. 2013;112:1142-7.
- [14] Kotecha D, Piccini JP. Atrial fibrillation in heart failure: what should we do? *Eur Heart J*. 2015;36:3250-7.
- [15] Guha K, McDonagh T. Heart failure epidemiology: European perspective. *Current cardiology reviews*. 2013;9:123-7.
- [16] van Deursen VM, Urso R, Laroche C, Damman K, Dahlstrom U, Tavazzi L, et al. Co-morbidities in patients with heart failure: an analysis of the European Heart Failure Pilot Survey. *Eur J Heart Fail*. 2014;16:103-11.
- [17] Kirchhof P, Ammentorp B, Darius H, De Caterina R, Le Heuzey JY, Schilling RJ, et al. Management of atrial fibrillation in seven European countries after the publication of the 2010 ESC Guidelines on atrial fibrillation: primary results of the PREvention of

thromboembolic events--European Registry in Atrial Fibrillation (PREFER in AF). *Europace*. 2014;16:6-14.

[18] Patti G, Lucerna M, Pecun L, Siller-Matula JM, Cavallari I, Kirchhof P, et al. Thromboembolic Risk, Bleeding Outcomes and Effect of Different Antithrombotic Strategies in Very Elderly Patients With Atrial Fibrillation: A Sub-Analysis From the PREFER in AF (PREvention of Thromboembolic Events-European Registry in Atrial Fibrillation). *Journal of the American Heart Association*. 2017;6: pii: e005657. doi: 10.1161/JAHA.117..

[19] Aho K, Harmsen P, Hatano S, Marquardsen J, Smirnov VE, Strasser T. Cerebrovascular disease in the community: results of a WHO collaborative study. *Bull World Health Organ*. 1980;58:113-30.

[20] Alpert JS, Thygesen K, Jaffe A, White HD. The universal definition of myocardial infarction: a consensus document: ischaemic heart disease. *Heart*. 2008;94:1335-41.

[21] Bassand JP, Hamm C. New European guidelines for the management of patients with unstable angina/non-ST-elevation myocardial infarction--what are the new and key messages. *Pol Arch Med Wewn*. 2007;117:391-3.

[22] Kirchhof P, Benussi S, Kotecha D, Ahlsson A, Atar D, Casadei B, et al. 2016 ESC Guidelines for the management of atrial fibrillation developed in collaboration with EACTS. *Eur Heart J*. 2016;37:2893-962.

[23] Kotecha D, Mohamed M, Shantsila E, Popescu BA, Steeds RP. Is echocardiography valid and reproducible in patients with atrial fibrillation? A systematic review. *Europace*. 2017.

[24] Dries DL, Exner DV, Gersh BJ, Domanski MJ, Waclawiw MA, Stevenson LW. Atrial fibrillation is associated with an increased risk for mortality and heart failure progression in patients with asymptomatic and symptomatic left ventricular systolic dysfunction: a retrospective analysis of the SOLVD trials. *Studies of Left Ventricular Dysfunction*. *J Am Coll Cardiol*. 1998;32:695-703.

[25] Loh E, Sutton MS, Wun CC, Rouleau JL, Flaker GC, Gottlieb SS, et al. Ventricular dysfunction and the risk of stroke after myocardial infarction. *N Engl J Med*. 1997;336:251-7.

[26] Hays AG, Sacco RL, Rundek T, Sciacca RR, Jin Z, Liu R, et al. Left ventricular systolic dysfunction and the risk of ischemic stroke in a multiethnic population. *Stroke*. 2006;37:1715-9.

[27] McMurray JJ, Ezekowitz JA, Lewis BS, Gersh BJ, van Diepen S, Amerena J, et al. Left ventricular systolic dysfunction, heart failure, and the risk of stroke and systemic embolism in patients with atrial fibrillation: insights from the ARISTOTLE trial. *Circulation Heart failure*. 2013;6:451-60.

[28] Pullicino PM, Halperin JL, Thompson JL. Stroke in patients with heart failure and reduced left ventricular ejection fraction. *Neurology*. 2000;54:288-94.

[29] Banerjee A, Taillandier S, Olesen JB, Lane DA, Lallemand B, Lip GY, et al. Ejection fraction and outcomes in patients with atrial fibrillation and heart failure: the Loire Valley Atrial Fibrillation Project. *Eur J Heart Fail*. 2012;14:295-301.

[30] Sandhu RK, Hohnloser SH, Pfeffer MA, Yuan F, Hart RG, Yusuf S, et al. Relationship between degree of left ventricular dysfunction, symptom status, and risk of embolic events in patients with atrial fibrillation and heart failure. *Stroke*. 2015;46:667-72.

[31] Senoo K, Lip GY, Lane DA, Buller HR, Kotecha D. Residual Risk of Stroke and Death in Anticoagulated Patients According to the Type of Atrial Fibrillation: AMADEUS Trial. *Stroke*. 2015;46:2523-8.

[32] McDonagh TA, Morrison CE, Lawrence A, Ford I, Tunstall-Pedoe H, McMurray JJ, et al. Symptomatic and asymptomatic left-ventricular systolic dysfunction in an urban population. *Lancet*. 1997;350:829-33.

[33] Scherbakov N, Haeusler KG, Doehner W. Ischemic stroke and heart failure: facts and numbers. *ESC Heart Failure*. 2015;2:1-4.

- [34] Sartipy U, Dahlstrom U, Fu M, Lund LH. Atrial Fibrillation in Heart Failure With Preserved, Mid-Range, and Reduced Ejection Fraction. *JACC Heart failure*. 2017;5:565-74.
- [35] Olsson LG, Swedberg K, Ducharme A, Granger CB, Michelson EL, McMurray JJ, et al. Atrial fibrillation and risk of clinical events in chronic heart failure with and without left ventricular systolic dysfunction: results from the Candesartan in Heart failure-Assessment of Reduction in Mortality and morbidity (CHARM) program. *J Am Coll Cardiol*. 2006;47:1997-2004.
- [36] Eapen ZJ, Greiner MA, Fonarow GC, Yuan Z, Mills RM, Hernandez AF, et al. Associations between atrial fibrillation and early outcomes of patients with heart failure and reduced or preserved ejection fraction. *Am Heart J*. 2014;167:369-75 e2.
- [37] Thavendiranathan P, Grant AD, Negishi T, Plana JC, Popovic ZB, Marwick TH. Reproducibility of echocardiographic techniques for sequential assessment of left ventricular ejection fraction and volumes: application to patients undergoing cancer chemotherapy. *J Am Coll Cardiol*. 2013;61:77-84.
- [38] Bellenger NG, Burgess MI, Ray SG, Lahiri A, Coats AJ, Cleland JG, et al. Comparison of left ventricular ejection fraction and volumes in heart failure by echocardiography, radionuclide ventriculography and cardiovascular magnetic resonance; are they interchangeable? *Eur Heart J*. 2000;21:1387-96.

TABLES:**Table 1.** Patient demographic data according to heart failure subgroups.

	HFrEF	HFmEF	HFLpEF	HFHpEF	no HF	p between HF groups
N	458	525	308	308	4571	
Age (years)	72	73	75	76	71	<0.0001
BMI	27.4	28.0	27.9	28.1	28.0	0.0926
Male (%)	76	67	59	45	59	<0.0001
Smoking (%)	49	46	38	31	39	<0.0001
Prior stroke (%)	10	11	10	8	8	0.7993
Coronary heart disease (%)	46	41	39	27	18	<0.0001
Prior stenting (%)	24	17	11	11	8	<0.0001
Prior myocardial infarction (%)	29	22	16	8	7	<0.0001
Peripheral arterial disease (%)	7.4	7.6	7.9	5.0	3.5	0.4515
Chronic kidney disease (%)	29	16	20	22	10	<0.0001
Chronic obstructive pulmonary disease (%)	19	17	22	16	9	0.1189
Systole blood pressure (mmHg) at baseline (mean)	123	130	129	133	133	<0.0001
Diastolic blood pressure (mmHg) at baseline (mean)	74	78	76	76	78	<0.0001
Heart rate (beats/min) at baseline (mean)	82	81	80	78	78	0.0180
Prior major bleeding event (%)	5.2	3.8	9.1	7.5	3.4	0.0096
HASBLED score (mean)	2.3	2.2	2.5	2.3	1.9	0.0696
Paroxysmal AF (%)	17.9	21.0	21.1	19.2	33.8	0.6090
Valvular heart disease (moderate or severe)	35.2	26.9	26.6	24.0	13.3	0.0027
Antithrombotic therapies (%):						
No therapy	3.7	4.6	4.6	2.9	6.7	0.6336
Oral anticoagulant (VKA or VKA & antiplatelet or NOAC)	88	87	88	94	81	0.0189
VKA	65	67	75	75	66	0.0027
VKA plus antiplatelet	18.6	13.1	7.5	9.1	8.9	<0.0001
NOAC	4.6	5.5	5.5	9.4	6.1	0.0397
NOAC plus antiplatelet	0.7	1.5	0.7	1.3	1.1	0.4847
Antiplatelet only	8.5	8.2	7.8	3.3	12.6	0.0261

Legend: HFrEF: heart failure with reduced ejection fraction; HFmrEF: HF with mid-range EF; HFLpEF: HF with lower preserved ejection fraction; HFHpEF : HF with higher preserved ejection fraction; BMI: body mass index; NOAC: non-vitamin K oral anticoagulants; VKA: vitamin K oral anticoagulants; TIA: transient ischemic attack. Data are presented as n, mean or percentages as appropriate. p value for trend.

Table 2. Mean CHA₂DS₂-VASc score and its components according to heart failure subgroups.

	HFrEF	HFmEF	HFLpEF	HFHpEF	no HF	p between HF groups
CHA2DS2-VASc score	4.1	4.4	4.5	4.7	3.0	<0.0001
Points 0 (%)	0	0	0	0	6.6	0.2458
Points 1 (%)	4.9	5.4	1.7	1	12.9	0.0007
Points ≥ 2 (%)	95.1	94.6	98.3	99.0	80.5	0.0016
Congestive heart failure (%)	100	100	96.0	94.3	0.0	<0.0001
Hypertension (%)	65.9	71.4	80.4	79.3	71.2	<0.0001
Age 65–74 years (%)	33.7	30.9	30.4	26.7	33.9	0.2395
Age ≥ 75 years (%)	43.5	49.7	56.6	63.0	41.9	<0.0001
Diabetes mellitus (%)	26.4	31.6	29.6	32.3	19.1	0.2437
Prior stroke/TIA/thromboembolic event (%)	16.2	18.8	15.5	15.7	15.2	0.5302
Vascular disease (%)	43.7	33.3	30.1	25.3	17.5	<0.0001
Female gender (%)	23.7	33.7	40.1	55.3	40.9	<0.0001

Legend: HFrEF: heart failure with reduced ejection fraction; HFmrEF: HF with mid-range EF; HFLpEF: HF with lower preserved ejection fraction; HFHpEF : HF with higher preserved ejection fraction; TIA: transient ischemic attack. p value for trend.

FIGURES

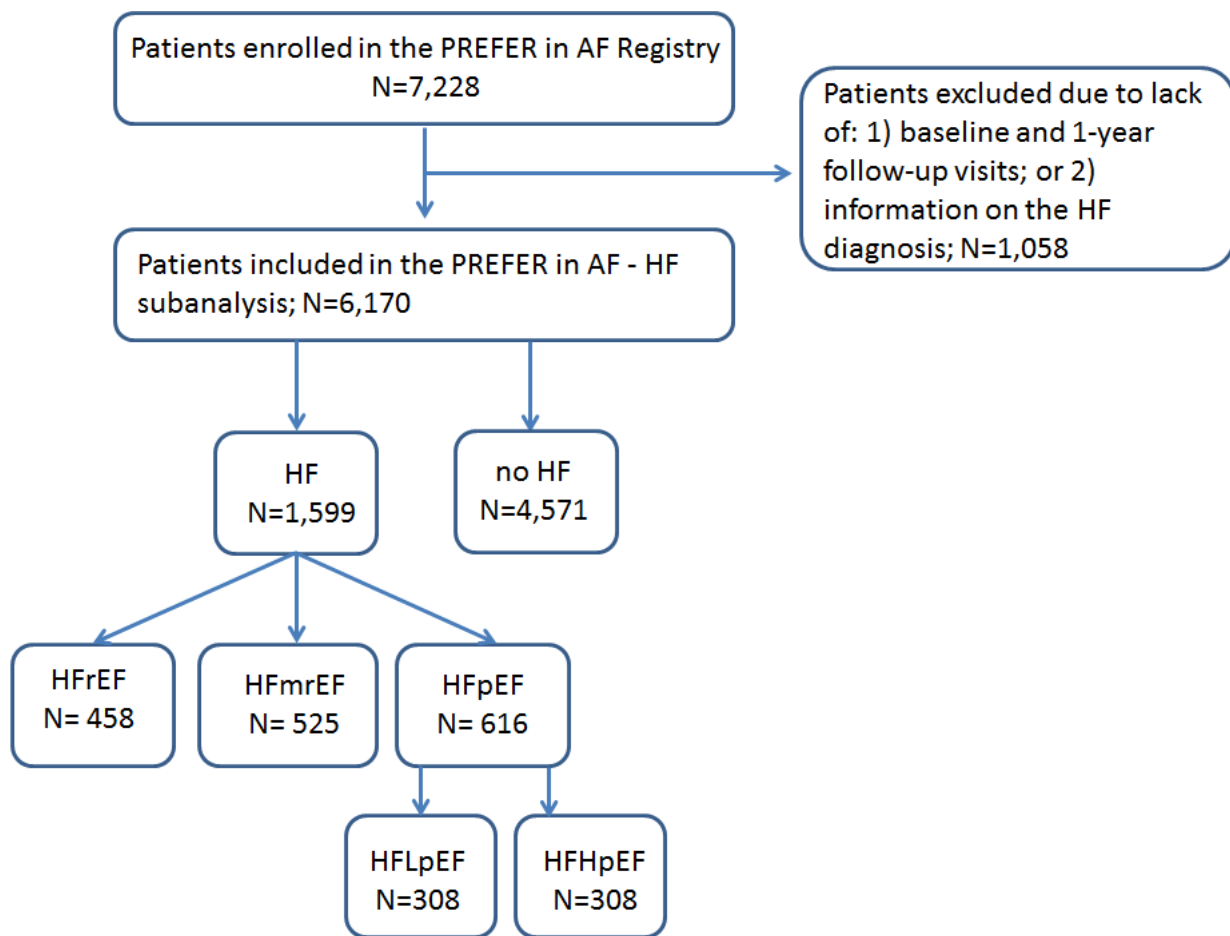


Figure 1. Flow of patients in the PREFER in AF – HF substudy. HFrEF: heart failure with reduced ejection fraction; HFmrEF: HF with mid-range EF; HFpEF: HF with preserved ejection fraction; HFLpEF: HF with lower preserved ejection fraction; HFHpEF: HF with higher preserved ejection fraction.

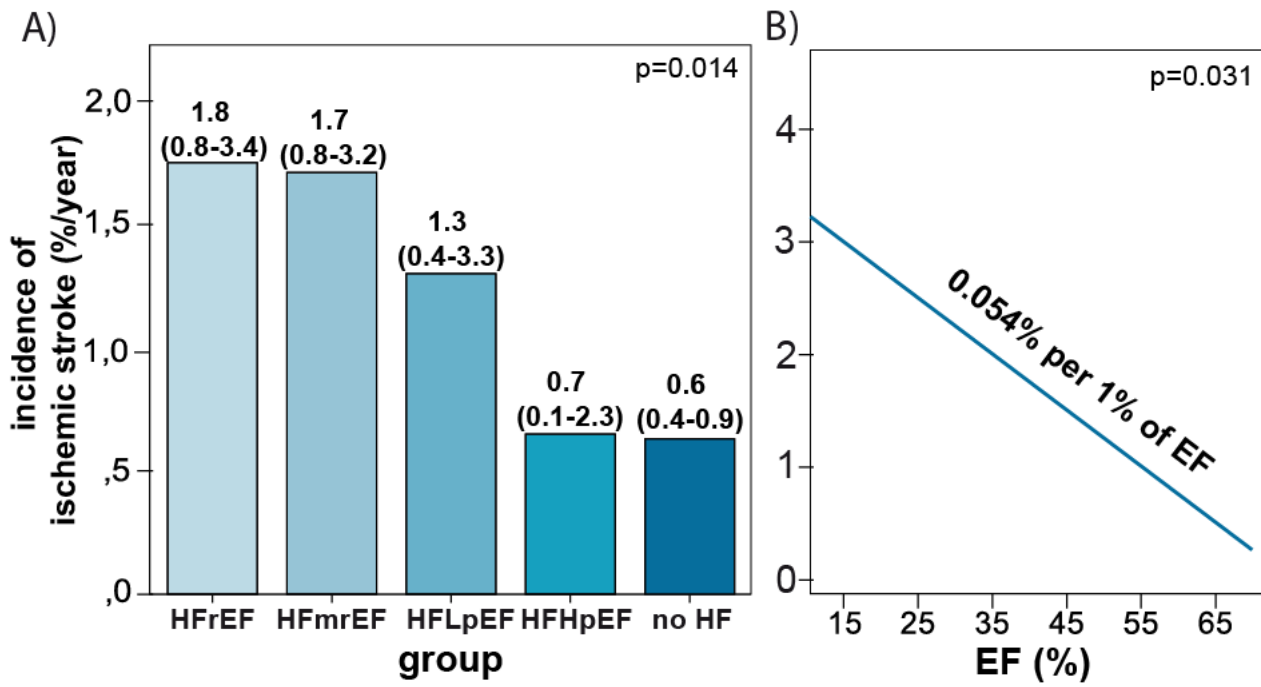


Figure 2. A) Annual incidence (mean and 95% confidence intervals, (CI)) of ischemic stroke; p value for trend; B) Linear regression model for the association between the incidence of ischemic stroke and ejection fraction (EF).

DISCLOSURES:

Authors have participated in a scientific development program sponsored by Daiichi Sankyo: the Thrombosis Exchange Meeting in AF (TEAM in AF).

Jolanta M. Siller-Matula: lecture or consultant fees from AstraZeneca, Daiichi Sankyo, Eli Lilly, Bayer and research grant from Roche Diagnostics; Ladislav Pecen: consultant fees from Daiichi-Sankyo, SOTIO, Beckman Coulter, Novartis; Giuseppe Patti: speaker/consultant/advisory board for Bayer, Boehringer-Ingelheim, BMS-Pfizer, Daiichi Sankyo, Astra Zeneca, Sigma-Tau, Malesci, PIAM, Amgen, Sanofi and MSD; Markus Lucerna: employee of Daiichi Sankyo; Paulus Kirchhof: PK receives research support from European Union, British Heart Foundation, Leducq Foundation, Medical Research Council (UK), and German Centre for Cardiovascular Research, from several drug and device companies active in atrial fibrillation, and has received honoraria from several such companies. PK is listed as inventor on two patents held by University of Birmingham (Atrial Fibrillation Therapy WO 2015140571, Markers for Atrial Fibrillation WO 2016012783). Maciej Lesiak: speaker's honoraria from AstraZeneca; Kurt Huber: Honoraria: BOEHRINGER INGELHIEM, BAYER, BRISTOL MYERS SQUIBB, DAIICHI SANKYO, PFIZER; Freek W.A. Verheugt: honoraria for speaker fees and consultancy honoraria from AstraZeneca, Medtronic, Bayer Healthcare, Boehringer-Ingelheim, BMS/Pfizer and Daiichi-Sankyo; Irene M. Lang: has relationships with drug companies including AOPOrphan Pharmaceuticals, Actelion, Bayer, Astra-Zeneca, Servier, Cordis, Medtronic, GSK, Pfizer and Ferrer, in the previous 3 years; Giulia Renda: consultant and speaker fees from: Bayer, Boehringer-Ingelheim, Daiichi-Sankyo; Renate B. Schnabel: None; Rolf Wachter: research grants from Boehringer Ingelheim, European Union and Bundesministerium für Bildung und Forschung (BMBF). Honoraria: Bayer, Bristol-Myers-Squibb, Boehringer Ingelheim, CVRx, Medtronic, Novartis, Pfizer, Sanofi, Servier outside the submitted work. Reimbursement for travel and accommodation costs from Daiichi-Sankyo; Dipak Kotecha: Research grants from Menarini; Jean-Marc Sella: lecture or consultant fees from Boehringer Ingelheim, Bayer, Bristol Myers-Squibb/Pfizer and Sanofi; Miklos Rohla: none; Fabrizio Ricci: none; Raffaele De Caterina: reports that – unrelated to this work – his institution received research grant support from Boehringer-Ingelheim, Bayer, Bristol-Myers Squibb/Pfizer and Roche; and he received personal honoraria for lectures and/or consulting from Boehringer-Ingelheim, Bayer, Bristol-Myers Squibb/Pfizer, Daiichi Sankyo, Lilly, AstraZeneca, Merck, Lilly and Novartis.

Author's contribution:

Jolanta M. Siller-Matula: conception and study design, interpretation of data, manuscript drafting; Ladislav Pecen: statistical analyses, interpretation of data, critical revision of the manuscript; Giuseppe Patti: interpretation of data, results discussion, critical revision of the manuscript; Markus Lucerna: interpretation of data, results discussion, critical revision of the manuscript; Paulus Kirchhof: interpretation of data, results discussion, critical revision of the manuscript; Maciej Lesiak: interpretation of data, results discussion, critical revision of the manuscript; Kurt Huber: interpretation of data, results discussion, critical revision of the manuscript; Freek W.A. Verheugt: interpretation of data, results discussion, critical revision of the manuscript; Giulia Renda: interpretation of data, results discussion, critical revision of the manuscript; Irene M. Lang: interpretation of data, results discussion, critical revision of the manuscript; Renate B. Schnabel: interpretation of data, results discussion, critical revision of the manuscript; Rolf Wachter: interpretation of data, results discussion, critical revision of the manuscript; Dipak Kotecha: interpretation of data, results discussion, critical revision of the manuscript; Marc Sellal: interpretation of data, results discussion, critical revision of the manuscript; Miklos Rohla: interpretation of data, results discussion, critical revision of the manuscript; Fabrizio Ricci: interpretation of data, results discussion, critical revision of the manuscript; Raffaele De Caterina: conception and study design, interpretation of data, manuscript drafting;